



Between-herd prevalence of *Mycoplasma bovis* in bulk milk in Flanders, Belgium

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ABSTRACT

Mycoplasma bovis (*M. bovis*) is a highly infectious pathogen of cattle causing pneumonia, polyarthritis, otitis, and less frequently, subcutaneous abscesses, abortions and meningitis.

Ineffective drugs treatments, culling of infected cows and loss of milk production can lead to significant economic loss on dairy farms. The early detection of cows excreting *M. bovis* bacteria to prevent mastitis outbreaks is warranted. Reports suggest that the risk of *M. bovis* mastitis is higher in larger dairy herds.

The objective of this study is to estimate the herd-level prevalence of *M. bovis* in Flanders, Belgium by culturing bulk tank milk samples taken from dairy farms.

Three bulk tank milk samples per dairy herd were taken over four weeks, with collection intervals of two weeks. Culturing was done after pre-incubation using modified Hayflicks media to increase the chances of recovery of bacteria. For the identification of *M. bovis*, tDNA intergenic spacer PCR was used.

In three herds (1.5%) of the 200 herds sampled, *M. bovis* was isolated from one of the three consecutive bulk tank milk samples. We conclude that in Flanders in 2009 at least 1.5% of the dairy herds had one or more cows excreting *M. bovis* in the milk.

The frequent monitoring of bulk tank milk to detect the presence of *M. bovis*, especially in expanding herds on farms that often purchase replacement animals, should be encouraged in order to detect the presence of *M. bovis* and to monitor the success of control procedures following an outbreak of mycoplasmal mastitis in the herd.

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Mycoplasma bovis is a highly infectious pathogen of cattle, causing pneumonia, polyarthritis, otitis, and, less frequently, subcutaneous abscesses, abortions, and meningitis (Nicholas and Ayling, 2003). In addition, it is the most important agent of outbreaks of mycoplasmal mastitis in dairy cows (Gonzalez and Wilson, 2003). *Mycoplasma* spp. lack a typical cell wall, and so are not affected by many of the commercially available antimicrobial drugs, which act by interfering with cell wall synthesis (Bushnell, 1984).

Over the last decade, *M. bovis* isolates have developed an acquired resistance to a wide range of commonly used antibiotics such as macrolides and tetracyclines (Nicholas et al., 2008). Intramammary infections with *M. bovis* are difficult to treat successfully even if the antimicrobials used show good *in vitro* activity against the agent (Ayling et al., 2000). Unsuccessful therapy, culling of infected cows and loss of milk production can lead to significant economic loss on a dairy farm (Nicholas and Ayling, 2003). The early

detection of cows excreting *M. bovis* to prevent mastitis outbreaks is warranted.

Recently, several cases of clinical and subclinical mastitis caused by *Mycoplasma* spp. in Belgian dairy herds have been reported (personal communication, Milk Control Centre Flanders, Lier, Belgium). Also, the number of milk samples submitted to the central milk quality laboratory (Milk Control Centre Flanders, Lier, Belgium) for bacteriological culturing for *M. bovis* increased from zero in 2007 to 553 in 2008 (a combination of bulk milk samples and individual cow milk samples). Nearly 9% ($n = 48$) of all samples were culture-positive (Annual Report 2008, Milk Control Centre Flanders, Lier, Belgium). No information is available on the between-herd prevalence of cows excreting *M. bovis* bacteria in Flanders, Belgium.

The culture of bulk tank milk samples is a valuable procedure for screening and surveillance of mastitis-causing pathogens at the herd level, in particular for the detection of cows excreting *Streptococcus agalactiae* and *M. bovis* (Jasper et al., 1979; Bushnell, 1984; Gonzalez et al., 1986; Gonzalez et al., 1992; Jayarao and Wolfgang, 2003).

The objective of this study was to estimate the herd-level prevalence of *M. bovis* in Flanders, Belgium, by means of culturing of bulk tank milk samples.

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Table 1

Bulk tank milk samples required and numbers tested during a survey for *Mycoplasma bovis*, to detect a between-herd prevalence of 5% (with 95% confidence), proportionally to the total number of dairy herds per province.

Province	Recorded no. dairy herds in Flanders	No. dairy herds required	No. dairy herds included
Antwerpen	1189	37	38
Limburg	594	19	19
Oost-Vlaanderen	1752	55	56
Vlaams Brabant	407	13	13
West-Vlaanderen	2346	74	75
Total	6287	197	200 ^a

^a One farm stopped delivering milk, was only sampled once, and omitted from the results.

The sample size required to estimate the prevalence of *Mycoplasma* spp. – infected herds accurately was calculated using Win Episcope 2.0 (Thrusfield et al., 2001). The target population was 6287 Flemish dairy producers with an expected prevalence of *M. bovis* of 5% ($\pm 3\%$), and a 95% confidence interval. Based on this calculation, the suggested adjusted sample size was 197 herds. In the 2009 year, 201 herds were selected randomly in Flanders in proportion to the total number of dairy farmers in each of the five Provinces for this study. (Table 1).

Bulk milk samples were collected through routine sampling as currently performed when milk is collected as part of the legal requirements for milk quality control procedures by the Milk Control Centre Flanders, Lier, Belgium. The milk samples were immediately stored at 4 °C and transported under cooled conditions (at 4 °C) to the laboratory for bacteriological analysis the next day. Three bulk milk samples per herd were collected and analysed over four weeks, with collection intervals of two weeks. Culturing was performed as described by the National Mastitis Council after pre-incubation using modified Hayflicks media to increase the recovery rates of the bacteria (Hogan et al., 1999). For identification of *M. bovis*, tDNA intergenic spacer PCR was used (Stakenborg et al., 2005).

One of the 201 selected farms stopped delivering milk during the study and was sampled only once (with a negative culture result). In the remaining 200 herds, *M. bovis* was isolated from one of the three consecutive bulk tank milk samples taken from 3 herds (1.5%). All culture-positive samples were positive at the first reading (three days after the commencement of incubation).

The between-herd prevalence of *M. bovis* in bulk milk ranges between 1% and 8% in the USA (Fox et al., 2003); is 5.4% in Greece (Filiosis et al., 2007), and is nil in New Zealand (McDonald et al., 2009). False-negatives may occur, suggesting the between-herd prevalence in Flanders, Belgium may be higher than the 1.5% prevalence found in this study.

Infected cows may excrete the organisms in low numbers (Gonzalez et al., 1986) or intermittently, and so may not be isolated on culture (Jasper et al., 1979; Bushnell, 1984; Biddle et al., 2003). Additionally, milk from *M. bovis* infected cows in large herds will be diluted in the total herd milk. Dairy producers also often withhold abnormal milk from the milk tank (Jasper et al., 1979; Thomas et al., 1981; Gonzalez et al., 1992).

Reports suggest that the risk of mastitis caused by *Mycoplasma* spp. is higher in large herds, presumably because cows and heifers are purchased more frequently either to maintain or expand the existing herd. (Thomas et al., 1981; Fox et al., 2003). During the last 20 years, the size of the dairy herd increased in Flanders, Belgium as illustrated by the increasing average volume of milk quota per farm (Annual Report 2010, Confederation of the Belgian Dairy Industry, Belgium). This increase in herd size is mainly driven by

the acquisition of cows and heifers from other herds, a key risk factor for the introduction of *M. bovis* into a dairy herd (Jasper et al., 1979; Gonzalez and Wilson, 2003).

As well, the average bulk milk somatic cell count in Flanders has increased since 1999, indicating that more attention to udder health management by farmers is required. Both of these observations indicate that the risk of *M. bovis* infection in a dairy herd has increased.

Our conclusion from the study is that in Flanders, Belgium in 2009, at least 1.5% of dairy herds had one or more cows excreting *M. bovis* in the milk. Frequent monitoring of bulk tank milk (especially on farms that purchase replacement animals) should be encouraged in order to screen for and detect the presence of *M. bovis* and to monitor the success of control procedures on the farm following an outbreak of mycoplasmal mastitis.

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